REMARKS

In the Office Action dated September 7, 2005, claims 33, 34 and 36-45 are pending. Claims 42-45 are withdrawn from consideration as directed to an invention that is allegedly independent or distinct from the invention originally claimed. Claims 33 and 34 are rejected under 35 U.S.C. §112, second paragraph, as allegedly incomplete for omitting essential steps. Claims 36-41 are rejected under 35 U.S.C. §112, second paragraph, allegedly because the metes and bounds of the claimed "vitrified" cell and composition are vague and unclear. Claims 39-41 are rejected under 35 U.S.C. §112, first paragraph, for allegedly failing to satisfy the written description requirement. Claims 36-38 are rejected under 35 U.S.C. §102(b) as anticipated by Thomson et al. (*Science* 282:1145-1147, 1998). Claims 36-38 are also rejected under 35 U.S.C. §102(e) as allegedly anticipated by Thomson (U.S. Patent No. 6,200,806). Claims 33 and 34 are rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Thomson et al. (*Science* or US Patent 6,200,806) and Vajta et al. (*Acta Vet Scan* 1997 or *Mol Reprod Dev* 1998).

This Response addresses each of the Examiner's rejections. Applicants respectfully submit that the present application is in condition for allowance. Favorable consideration of all pending claims is therefore respectfully requested.

Applicants first respectfully submit that claims 33, 34 and 36-45 are canceled without prejudice; and new claims 46-54 are added. New claims 46-54 are drawn to methods for preserving pluripotent human embryonic stem cells (hES) cells for continued storage and propagation by subjecting the hES cells to a process of vitrification. Support for these claims is found in the specification, e.g., page 24, lines 20-23, page 25, line 1, and page 30, lines 8-32. No new matter is introduced.

Claims 33 and 34 are rejected under 35 U.S.C. §112, second paragraph, as allegedly incomplete for omitting essential steps. The Examiner contends that the claims merely recite that the cells undergo vitrification and do not include an active step delineating the specific process of vitrification, or more specifically, the process of the OPS method.

Claims 33-34 have been canceled, rendering the rejection thereof moot. New independent claim 46 defines the specific process of the present method by including a step of incubating the sample of hES cells in a vitrification solution and a step of rapidly cooling the sample. As such, the rejection under 35 U.S.C. §112, second paragraph, for omitting essential steps, is overcome. Withdrawal of the rejection is therefore respectfully requested.

Claims 36-41 are rejected under 35 U.S.C. §112, second paragraph, as allegedly indefinite. Referring to "vitrified" hES cells recited in the claims, the Examiner contends that it is unclear as to how any form of cryopreservation would specifically affect the claimed cells. In other words, the Examiner contends that a cell frozen by one method may be the same as the cell frozen by another method. Therefore, the Examiner concludes that the metes and bounds of the claims directed to "vitrified" cells are vague and unclear.

Applicants respectfully submit that the Examiner's rejection appears only applicable to claims 36-38, which are drawn to a vitrified cell or a vitrified cell composition, but not to claims 39-41, which are drawn to methods of preserving cells. In any event, the rejection is rendered moot in view of the cancellation of claims 36-41. New claims 46-54 are not drawn to vitrified cells or compositions, but rather are directed to methods for preserving pluripotent hES cells by subjecting the hES cells to a define process of vitrification.

Accordingly, the rejection under 35 U.S.C. §112, second paragraph, relating to "vitrified" cells or compositions, is overcome. Withdrawal of the rejection is therefore respectfully requested.

Claims 39-41 are rejected under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Specifically, the claims recite vitrification buffers VS1 and VS2. The Examiner contends that the specification only supports VS1 and VS2 as specifically taught in the specification (i.e., with specific components at specific concentrations); and further, the specification only teaches the use of VS1 and VS2 in the context of the open pull straw method.

Applicants first respectfully submit that new claims 46-54 do not recite 'VS1" or "VS2", which is believed to render the written description rejection moot.

New claim 46 recites "a vitrification solution", which finds support in the specification. Specifically, the specification clearly discloses that the use of vitrification solutions in the process of vitrification (page 30, lines 15-16), and provides examples of two specific vitrificatin solutions, VS1 and VS2 (page 30, lines 16-21).

New claim 51 recites "a first vitrification solution" and "a second vitrification solution" with certain specified components. The sequential use of two vitrification solutions is disclosed in the specification at page 30, lines 21-25. Each of the recited components in the two vitrification solutions is also disclosed in the specification at page 30, lines 16-21.

New claims 52-54 further recite specific concentrations of certain components in the vitrification solution. The recited concentrations are also disclosed in the specification at page 30, lines 19-21.

Those skilled in the art would recognize that a key feature of the claimed method is to subject the hES cells to a rapid cooling vitrification process, and would appreciate that some modifications can be made (see page 30, lines 10-11), e.g., to the specific components and concentrations thereof of those vitrification solutions specifically exemplified in the specification.

Accordingly, it is respectfully submitted that new claims 46-54 are adequately described in the specification in full compliance with the written description requirement of 35 U.S.C. §112, first paragraph.

Claims 36-38 are rejected under 35 U.S.C. §102(b) as anticipated by Thomson et al. (*Science* 282:1145-1147, 1998). Claims 36-38 are also rejected under 35 U.S.C. §102(e) as anticipated by Thomson (US Patent 6,200,806-IDS Reference).

Claims 36-38 are directed to a vitrified hES cell or cell composition. The Examiner contends that these product claims are interpreted to cover a cryopreserved frozen hES cell prepared by any methodology of cryopreservation, absent a showing of distinct functional or structural features of the cells conferred by the vitrification process. Therefore, the Examiner concludes that the claims read on the cryopreserved cell lines disclosed by the Thomson references.

It is respectfully submitted that the §102 rejections of claims 36-38 are rendered moot in view of the cancellation of these claims. New claims 46-54 do not include claims directed to

vitrified cells or compositions. Accordingly, withdrawal of the §102(b) rejection and the §102(e) rejection is respectfully requested.

Claims 33 and 34 are rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Thomson (*Science* or US Patent 6,200,806) and Vajta et al. (*Acta Vet Scan* 1997 or *Mol Reprod Dev* 1998).

The Examiner acknowledges that Thomson does not teach a specific method of cryopreservation. However, the Examiner contends that it would be routine to use a method known in the art that was successfully used to preserve embryonic like cells. The Examiner contends that Vajta et al. teach that vitrification can be used for cryopreserving embryonic cells, and demonstrates the effectiveness of freezing porcine embryos by the OPS method. The Examiner is of the opinion that the cells of a developing embryo can be considered cells at a differentiated state, as recited in the present claims. The Examiner concedes that Vajta et al. do not specifically teach that the method disclosed therein should be practiced with human cells. However, the Examiner contends that it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made, to use the method of cryopreservation taught by Vajta et al. to preserve the hES cells as generally taught by Thomson.

In the first instance, Applicants respectfully submit that Thomson does not teach any specific method of cryopreservation. There are a variety of methods for cryopreserving cells, including many slow freezing methods as well as vitrification methods. Thomson does not suggest anywhere to crypreserve hES cells by a vitrification process. Therefore, Thomson does not provide those skilled in the art with a motivation to crypreserve hES cells by a vitrification process, much less with a reasonable expectation of success if a vitrification process is attempted on hES cells.

With respect to Vajta et al., this reference shows the use of vitrification in cryopreserving embryos and blastocysts. In the first instance, Applicants respectfully submit that an embryo or blastocyst has a different physical structure from hES cells from an *in vitro* culture that are preserved by the claimed methods. An embryo or blastocyst has a protective zona pellucida, in contrast to the cultured hES cells vitrified in the present invention which are exposed directly to the cryoprotectants. Prior to the filing of the present application, it was not predictable how hES cells would behave upon a direct exposure to cryoprotectants and whether a vitrification process would maintain the cells in a pluripotent state.

Furthermore, hES cells are a tissue culture artefact and are unlike any cell in any *in vivo* environment. To prepare hES cells, the trophectoderm is removed to access the ICM, therefore the "normal" embryonic environment is disrupted. That is, the ICM is now out of context with the trophectoderm and the native blastocyst environment has been destroyed. Any cells therefore remaining are not in their "natural environment". All media and subsequent supplements used to derive hES cells are *in vitro* "approximates" that have been found to be supportive of hES cells and do not mirror a normal *in vivo* environment. Hence, hES cells have characteristics that allow these cells to survive outside of a "natural" *in vivo* environment, distinct from cells with an embryo. On the basis of the artificial nature of hES cells alone, those skilled in the art would not reasonably expect that the methods of cryoprotection successfully applied on an embryo could be successfully applied to hES cells.

Moreover, those skilled in the art would recognize that at the time present application was filed, different types of cryopreservation are available, but are not equally applicable to all cells across the board. In particular, vitrification is not applicable to all cells, as discussed in Trounson et al. (1999, a copy of which is enclosed). The authors state that while some cells

types have been vitrified, high cryosurvival rates with vitrification remain a problem for many cell types. For example, lower cryosurvival after vitrification was found for human pancreatic islet cells, as compared with a conventional freezing method. Hence, Applicants respectfully submit that those skilled in the art could not have readily extrapolated the effectiveness of vitrification in preserving embryos and blastocysts, disclosed by Vajta et al., to other cells such as hES cells.

Applicants further respectfully submit that the new claims presently characterize the method as preserving hES cells as clumps. hES cells preserved according to the presently claimed methods are capable of propagation after thawing.

In contrast to the presently claimed methods, Thomson does not appreciate the need to cryopreserve hES cells in clumps, much less to cryopreserve hES cells in clumps by vitrification. In addition, Thomson does not show anywhere that the hES cells cryopreserved are able to propagate after the cells are thawed.

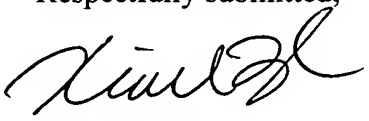
As to Vajta et al., the embryos vitrified contain 4 to 8 cells. These cells are destined to divide and differentiate upon thawing. In contrast, the claimed methods preserve hES cells, which are in a pluripotent state prior to vitrification, and remain in the pluripotent state without differentiation and are capable of propagation after thawing. See Example 6 on page 37 of the specification.

In view of the foregoing, Applicants respectfully submit that the references, either taken singularly or in combination, do not provide the requisite motivation to those skilled in the art to apply vitrification to hES cells, as presently claimed, or the requisite reasonable expectation of success if one were to attempt to apply vitrification to hES cells. Accordingly, Applicants respectfully submit that the claimed methods are not obvious in view of the cited

combination of the references. Withdrawal of the §103 rejection based on Thomson and Vajta et al. is overcome.

In view of foregoing amendments and remarks, it is firmly believed that the subject application is in condition for allowance, which action is earnestly solicited.

Respectfully submitted,



Xiaochun Zhu
Registration No. 56,311

Scully, Scott, Murphy & Presser, P. C.
400 Garden City Plaza, Suite 300
Garden City, New York 11530
(516) 742-4343
XZ:ab

Enc.: Trounson et al. (1999)

The First World Congress On: Controversies in Obstetrics, Gynecology & Infertility
Prague, Czech Republic - 1999

What Can We Expect from Thawing Gametes?

A. Trounson, L. Kuleshova and L. Gianaroli*

Centre for Early Human Development, Monash Institute of Reproduction and Development Monash University, Clayton, Victoria, Australia

** S.I.S.M.E.R., Reproductive Medicine Unit, Bologna, Italy*

Cryopreservation of cells and tissues has been applied for many decades with variable success. In the case of cell lines, the survival of a few percent of frozen cells will allow the re-establishment of cell cultures, particularly for robust cell types. Success of sperm cryopreservation is also based on the presence of many sperm cells (usually 4 to 25 x 106 sperm in each insemination straw) and the survival of a reasonable percentage of these sperm (10-50%). The demand for very high survival rates for human embryos has resulted in more refined and defined freezing methods involving very slow cooling rates during the ice formation phase (equilibration cooling). This enables embryo survival rates to be higher than 50% (often approaching 80%) and the application of embryo cryopreservation in clinical IVF. Implantation rates of surviving embryos varies from 8 to 20% and may not be very different to that of non-frozen embryos (1,2). Under these circumstances there is not much inducement to adopt new cryopreservation techniques such as vitrification for human embryos, although it has been reported to be successful. High concentrations of ethylene glycol (40%) and the polymer Ficoll (18%) together with 0.3 M (10%) sucrose have been used for cryopreservation of early cleavage stage human embryos, and the production of a set of healthy twins (1 of 18 patients transferred embryos) (3). Other cell types have also been vitrified using high concentrations of permeating cryoprotectants including human cord blood hematopoietic cells (4), tissue containing nerve segments (from rat hind leg) (5), liver slices (6), and established cell culture lines (osteosarcoma and malignant fibrous histosarcoma) (7). However, reproducibility of high cryosurvival rates with vitrification remains a problem for many cell types. Langer et al. (8) found low cryosurvival after vitrification of human pancreatic islet cells compared with conventional freezing. Human corneal endothelial tolerance to the high levels of permeating cryoprotectants needed for vitrification is also low (9).

Success rates for cryopreserved human oocytes have been extremely disappointing and far below survival rates that are required for clinical application. The most advanced data for the conventional slow-cooling of human oocytes has been reported by Dr. E. Porcu (10) from the University of Bologna. Mature oocytes were frozen using a minor modification of the technique used for human embryos involving the permeating cryoprotectant 1,2-propanediol (11). Of 753 thawed oocytes, 7 babies (6 births) were born which is an overall success rate of less than 1%. This is not significantly different to that achieved with the original slow cooling techniques reported for human oocytes (12) that resulted in a small number of births

in the late 1980's (13, 14). The only advance in methodology is the use of intracytoplasmic sperm injection (ICSI) to achieve fertilization in thawed oocytes because it is argued that this overcomes the hardening of the zona pellucida that may be caused by premature cortical granule release during freezing of oocytes (15). The large scale studies of Porcu et al. (10) do not appear to show any substantial benefit of ICSI on the development of thawed oocytes and there are no controlled studies to support the increased developmental competence of thawed oocytes fertilized by ICSI rather than conventional IVF. Tucker et al. (16) also reported the outcomes of thawing 334 human oocytes frozen by basically the same method as Porcu et al. (10). Only 70 (21%) oocytes survived thawing and 25 developed to transferable embryos. Three viable pregnancies involving four advanced fetuses were established after embryo transfer to recipient patients (an overall success rate of around 1%). It has also been shown recently that sodium salts are damaging to mouse oocytes during these conventional slow cooling methods for freezing because sodium is the major ion involved in the solution effects which cause cryoinjury to cell membranes during dehydration and rehydration (17). Substitution of sodium with choline in the cryopreservation medium significantly improved oocyte survival and developmental rates after freezing and thawing. An attempt to vitrify human oocytes in the original vitrification solution used by Rall and Fahy (18), which is a mixture of the permeating cryoprotectants (dimethyl sulphoxide, propanediol and polyethylene glycol plus acetamide), resulted in 9/20 (45%) fertilized oocytes but no further cleavage and development (19).

Recently we have shown that human oocytes can be vitrified successfully in low toxicity solutions of ethylene glycol, sucrose and human serum albumin. In the initial preliminary studies (20) of a small number of thawed oocytes, 67% survived vitrification intact, and 80% of these surviving oocytes fertilized and developed to 6- to 10-cell embryos by day 3. When three embryos were transferred to three patients, a single pregnancy and birth of a normal child resulted. Further studies have now been undertaken at the SISMER IVF Clinic, Bologna and further pregnancies have been achieved. Human oocytes have also been vitrified in 5.5 M ethylene glycol and 1 M sucrose on microscope copper grids (21). Oocytes survived vitrification and fertilized at relatively high rates (38-71%, depending on group treatment). The embryos developed to 4- to 8-cells and to blastocysts in some treatment groups. These new data will be discussed and the potential applications explored in more detail.

References

(1) TROUNSON AO, SHAW JM. Embryo cryopreservation. Reprod. Med. Review 1: 179-188, 1992.

(2) TROUNSON AO, BONGSO A. Fertilization and development in humans. Curr. Topics Devel. Biol. 32: 59-101, 1996.

(3) MUKAIDA T, WADA S, ET AL. Vitrification of human embryos based on the assessment of suitable conditions for 8-cell mouse embryos. Hum. Reprod. 13: 2874-2879, 1998.

(4) KURATA H, TAKAKUWA K, TANAKA K. Vitrification of hematopoietic progenitor cells obtained from human cord blood. Bone Marrow Transplantation 14: 261-263, 1994.

(5) DECHERCHI P, LAMMARIBARREAULT N, ET AL. CNS axonal regeneration within peripheral nerve grafts cryopreserved by vitrification - cytological and functional aspects. Cryobiology 34: 214-239, 1997.

(6) EKINS S, WILLIAMS JA, ET AL. Xenobiotic metabolism in rat, dog, and human precision-cut liver slices, freshly isolated hepatocytes, and vitrified precision-cut liver slices. Drug Metabol. Disposition 24: 990-995, 1996.

(7) ALI J. Highly efficient ultrarapid cryopreservation of established cell lines by vitrification with VS14. Med. Sci. Research 24: 837-838, 1996.

(8) LANGER S, LAU D, ET AL. Viability and recovery of frozen-thawed human islets and in vivo quality control by xenotransplantation. J. Mol. Medic. 77: 172-174, 1999.

(9) BOURNE WM, NELSON LR. Human corneal studies with a vitrification solution containing dimethyl sulfoxide, formamide, and 1,2-propanediol. Cryobiology 31: 522-530, 1994.

(10) PORCU E, FABBRI R, ET AL. Birth of six healthy children after intracytoplasmic sperm injection of cryopreserved human oocytes. Abstracts of ESHRE, G□teborg, Hum. Reprod. 124, 1998.

(11) GOOK DA, OSBORN SM, ET AL. Cryopreservation of mouse and human oocytes using 1,2-propanediol and the configuration of the meiotic spindle. Hum. Reprod. 8: 1101-1109, 1993.

(12) TROUNSON A. Preservation of human eggs and embryos. Fertil. Steril. 46: 1-12, 1986. (13) CHEN C. Pregnancy after human oocyte cryopreservation. Lancet 884-886, 1986.

(14) VAN UEM JFHM, SIEBZEHNRUBL ER, ET AL. Birth after cryopreservation of unfertilised oocytes. Lancet 752-753, 1987.

(15) GOOK DA, OSBORN SM, ET AL. Parthenogenetic activation of human oocytes following cryopreservation using 1,2-propanediol. Hum. Reprod. 10: 654-658, 1995.

(16) TUCKER MJ, MORTON PC, ET AL. Clinical application of human egg cryopreservation. Hum. Reprod. 13: 3156-3159, 1998.

(17) STACHECKY JJ, COHEN J, WILLADSEN S. Detrimental effects of sodium during mouse oocyte cryopreservation. Biol. Reprod. 59: 395-400, 1998.

(18) RALL WF, FAHY GM. Ice-free cryopreservation of mouse embryos at -1960C by vitrification. Nature 313: 573-575, 1985.

(19) HUNTER JE, FULLER BJ, ET AL. Vitrification of human oocytes following minimal exposure to cryoprotectants; initial studies on fertilization and embryonic development. Hum. Reprod. 10: 1184-118, 1995.

(20) KULESHOVA L, GIANAROLI L, MAGLI C, FERRARETTI A, TROUNSON A. Case report: Birth following vitrification of a small number of human oocytes. Hum. Reprod. (In press) 1999.

(21) HONG SW, CHUNG HM, LIM JM, KO JJ, YOON TK, YEE B, CHA KY. Improved human oocyte development after vitrification: a comparison of thawing methods. Fertil. Steril. 72: 142-146, 1999.